

Trisomy-21 gene dosage overexpression of miRNAs results in the haploinsufficiency of specific target proteins

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Abbreviations: DS, down syndrome; Ts21, trisomy 21; Hsa21, human chromosome 21; miRNA, microRNA; 3'-UTR, 3'-untranslated region; AD, alzheimer's disease; miRISCs, miRNA-induced silencing complexes; LNA, locked nucleic acid; MeCP2, methyl-CpG-binding protein; RS, rett syndrome; AML, acute myeloid leukemia; HD, homozygous deletion; CDC2, cell division cycle 2; EGF, epidermal growth factor; AT₁R, angiotensin II type 1 receptor

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Down syndrome (DS) or Trisomy 21 (Ts21) is caused by the presence of an extra copy of all or part of human chromosome 21 (Hsa21) and is the most frequent survivable congenital chromosomal abnormality. Bioinformatic annotation has established that Hsa21 harbors more than 400 genes, including five microRNA (miRNA) genes (miR-99a, let-7c, miR-125b-2, miR-155 and miR-802). MiRNAs are endogenous, single-stranded, small non-coding RNA molecules that regulate gene expression by interacting with specific recognition elements harbored within the 3'-untranslated region (3'-UTR) of mRNAs and subsequently target these mRNAs for translational repression or destabilization. MiRNA expression profiling, miRNA RT-PCR and miRNA in situ hybridization experiments have demonstrated that Hsa21-derived miRNAs were overexpressed in fetal brain and heart specimens isolated from individuals with DS. We now propose that Ts21 gene dosage overexpression of Hsa21-derived miRNAs in DS individuals result in the decreased expression of specific target proteins (i.e., haploinsufficiency) that contribute, in part, to the DS phenotype.

Introduction

DS or Ts21 usually results from the failure of chromosome pairs to separate properly during cell division (i.e., nondisjunction).¹ In DS individuals nondisjunction usually occurs during meiotic division I and II (~95% of the time), is frequently

of maternal origin (~90% of cases) and is often associated with advanced maternal age.²⁻⁴ Although Ts21 can be divided into four categories according to the size of the triplicated genomic region (i.e., complete trisomy, partial trisomy, microtrisomy and single-gene duplication), the majority of DS individuals (~95%) have three complete copies of Hsa21.²⁻⁴ DS is the most frequent survivable congenital chromosomal abnormality occurring in approximately 750–1,000 live births with an estimated 5,000 infants born with DS each year in the USA.^{5,6}

The clinical presentation of DS is complex and variable. A constellation of over 80 clinical features have been described in DS patients, but cognitive impairments are one of the most constant features with varying degrees of penetrance.⁷⁻⁹ In addition to cognitive dysfunction, the phenotypes of DS include congenital heart defects, craniofacial abnormalities, gastrointestinal anomalies, childhood leukemia, altered immune responses and early-onset Alzheimer's disease.⁷⁻⁹ Interestingly, even though DS individuals have a high incidence of leukemia, the risk of developing most solid tumors (e.g., neural tumors and breast cancer) is markedly reduced in DS patients across all age groups.¹⁰⁻¹⁴ Additionally, persons with DS have significantly lower systolic and diastolic blood pressure than do individuals with cognitive impairments due to other causes.¹⁵⁻¹⁹ Further, DS patients have a reduced risk of vascular anomalies compared with the general population.²⁰ Finally, the prevalence of coronary artery disease is low in DS subjects and pathological studies

revealed decreased levels of atherosclerosis in these; therefore, it has been suggested that DS individuals may represent an atherosclerosis-free model.²¹⁻²⁵

While the genetic anomaly in DS has been identified to be a triplication of Hsa21, it is not known how this chromosomal anomaly causes DS characteristics. It is hypothesized that a “gene-dosage” effect resulting from the 50% increase in expression at the RNA level of the trisomic genes leads to an imbalance of critical genes, which in turn, initiates the DS phenotype.²⁶⁻²⁸ While the DS phenotype is most likely a multigenic condition, it is evident that only a specific subset of trisomic Hsa21 genes play a role in mediating the DS phenotype²⁸⁻³¹ and, almost certainly, DS results from the interaction between Hsa21 genes and Hsa21 genes interacting with other disomic genes.

Hsa21-Derived miRNAs

MiRNAs are a family of small, ~21-nucleotide long, nonprotein-coding RNAs that have emerged as key post-transcriptional regulators of gene expression.^{32,33} MiRNAs are processed from precursor molecules (pri-miRNAs), which are either transcribed from independent miRNA genes or are portions of introns of protein-coding RNA polymerase II transcripts. Pri-miRNAs fold into hairpin structures containing imperfectly base-paired stems and are subsequently endonucleolytically processed.^{32,33} In principle, the resulting miRNA duplex could give rise to two different mature miRNAs. However, in a manner similar to siRNA duplexes, only one strand is usually incorporated into miRNA-induced silencing complexes (miRISCs); the other strand is degraded (the complementary miRNA* strand).⁵⁸ Still, recent data suggest that both arms of the pre-miRNA hairpin can give rise to mature miRNAs.^{32,33,38}

Once the miRISC is assembled, the miRNA acts as an adaptor for this complex to specifically recognize and regulate particular mRNAs. Mature miRNAs recognize their target mRNAs by base-pairing interactions between nucleotides 2 and 8 of the miRNA (the seed region) and complementary nucleotides in the

3'-UTR of mRNAs. MiRISCs subsequently inhibit gene expression by targeting mRNAs for translational repression or destabilization.^{32,33}

Bioinformatic annotation has established that Hsa21 harbors more than 400 genes,^{34,35} including five microRNA (miRNA) genes (miR-99a, let-7c, miR-125b-2, miR-155 and miR-802, <http://www.mirbase.org/index.shtml>).³⁶ MiR-99a, let-7c and miR-125b-2 reside in the sense orientation within intron 6 of the *C21orf34* gene (intron number designation changes because of alternative *C21orf34* promoter utilization). The *C21orf34* gene is located at the beginning of q21.1 and the protein encoded by this gene has not been characterized. MiR-99a and let-7c are only 659 bp apart, whereas miR-125b-2 is located just over 50,000 bp downstream of let-7c. It is currently not known whether this genomic cluster is transcribed and regulated independent of the host gene and/or one another. MiR-155 resides within exon 3 of the spliced and polyadenylated non-coding B-cell integration cluster (BIC) gene, which is frequently targeted for retroviral insertion in chickens.³⁷ The BIC/miR-155 gene is located almost nine million base pairs downstream from the *C21orf34* gene at Hsa21 genomic position q21.2. Finally, miR-802 is located over 10.1 million base pairs downstream from the BIC/miR-155 gene in the antisense orientation within intron 1 of the *RUNX1* gene at position q22.11. Currently very little information is known regarding the expression distribution of this miRNA or the mechanisms by which it is regulated.

Experiments investigating the tissue specific distribution of the five Hsa21-derived miRNAs and their respective miRNA* species (probe sets are currently not available for miR-802*) demonstrated that miR-99a, let-7c and miR-125b-2 are abundantly expressed in a number of tissues including the DS relevant tissues, the brain and heart (Fig. 1). It is important to note that the expression levels of miR-125b-2 are an over-estimate due to cross-reactivity of qPCR probes with its miR-125b-1 ortholog.⁴¹ In contrast miR-155 and miR-802 are expressed at much lower levels with the highest expression

being in the thymus, the colon and small intestine, respectively (Fig. 1). Although the Hsa21-derived miRNA* species were detectable in all the tissues investigated, they were usually at least 100-fold less abundant than their non-star miRNA complement (data not shown) suggesting that unlike some recent studies investigating the functionality of miRNA* strands,^{32,33,38} the Hsa21 miRNA* species may not be biologically important.

Hsa21-Derived miRNAs' Expression in DS Tissues

Given that bioinformatic annotation has established that Hsa21 harbors five miRNA genes, it was hypothesized that Ts21 may result in a gene dosage overexpression of these miRNAs. This hypothesis was first tested in fibroblasts isolated from an individual with DS and from their unaffected monozygotic twin.³⁹ Twins were selected for study so that gene-expression differences could be attributed to only the supernumeracy of Hsa21 and not polymorphic variability in the rest of the genome. Importantly, these experiments demonstrated that miR-155 was more abundantly expressed in the fibroblasts isolated from the DS individual when compared with the unaffected twin;³⁹ the expression levels of the other four Hsa21-derived miRNAs were not determined.

To extend these results miRNA expression profiling experiments were performed utilizing RNA isolated from control and DS fetal hippocampus samples.⁴⁰ Of the 424 human mature miRNAs investigated, 10 were overexpressed and 22 were under-expressed by at least 50% in hippocampus specimens isolated from individuals with DS when compared with controls. Importantly, of the five Hsa21-derived miRNAs, miR-99a, let-7c and miR-155 were overexpressed in the profiling experiments. The values for miR-125b-2 were inconclusive and miR-802 was not profiled.⁴⁰

To validate the microarray studies, qPCR experiments were performed using primer sets specific for the five Hsa21-derived miRNAs. These experiments demonstrated that mature miR-99a, let-7c, miR-125b-2, miR-155 and miR-802

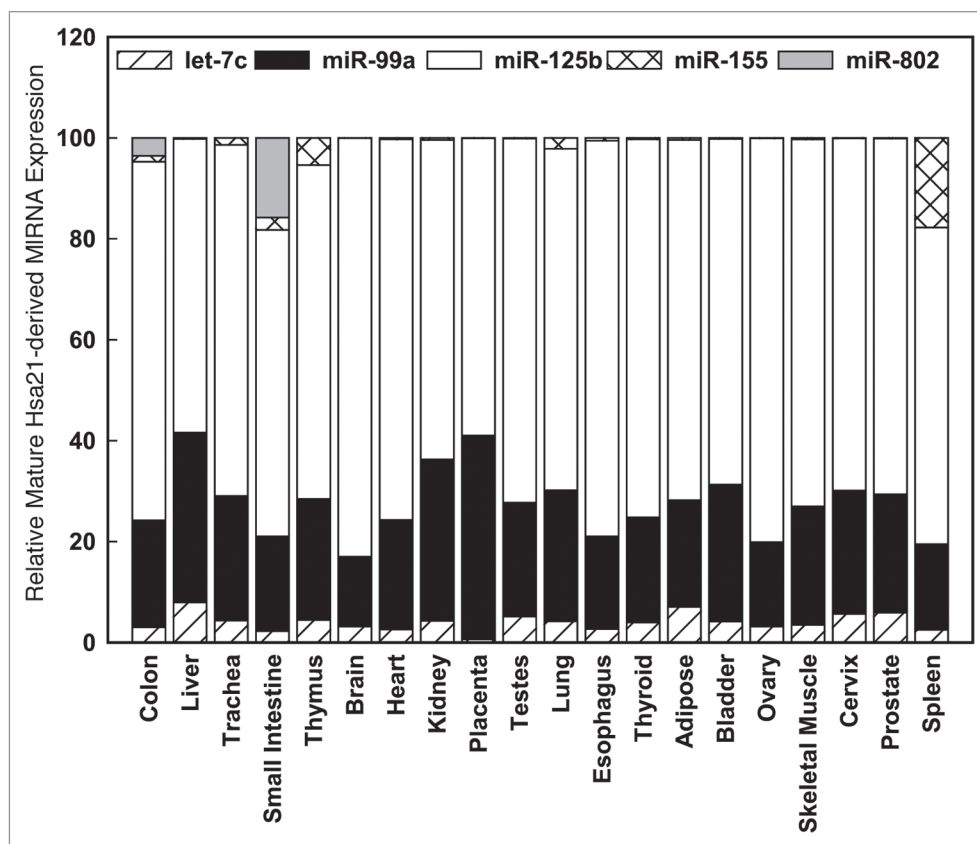


Figure 1. Relative expression of Hsa21-derived miRNAs in human tissues. Mature human let-7c, miR-99a, miR-125b, miR-155 and miR-802 were quantified utilizing TaqMan microRNA assay kits specific for each Hsa21-derived miRNA (Applied Biosystems, Foster City, CA) as previously described.^{40,42} The expression values were normalized to RNU48 for each tissue. Relative gene expression was calculated as $2^{-(CT_{miR-155} - CT_{RNU48})}$. The relative abundance of each Hsa-21 derived miRNA is shown as a percentage of total combined expression of Hsa-21 derived miRNAs for each tissue investigated.

were overexpressed by at least 50% in fetal hippocampus and heart samples obtained from DS individuals when compared with age- and sex-matched control specimens.⁴⁰ Hsa21-derived miRNA expression was also investigated in these samples by in situ hybridization experiments utilizing antisense miRNA locked nucleic acid (LNA) probes specific for each Hsa21-derived miRNA. Importantly, these experiments demonstrated that let-7c, miR-125b-2, miR-155 and miR-802 expressing neurons were increased by 10- to 15-fold and miR-99a expressing neurons were increased by ~50-fold in DS samples.⁴⁰ Finally, qPCR utilizing total RNA isolated from human prefrontal cortex specimens from brains of fetuses, infants/children, adolescents and adults with DS demonstrated that all five Hsa21-derived miRNAs were overexpressed by at least 50% in prefrontal cortex samples at all ages examined when compared with age- and sex-matched control brain specimens (Fig. 2).⁴²

Hsa21-Derived miRNA/mRNA Target Prediction

Given that Ts21 results in the gene dosage overexpression of Hsa21-derived miRNAs, we now hypothesize, as a consequence, the expression levels of specific target proteins would be decreased (i.e., haploinsufficiency), which in turn could contribute to the features of the DS phenotype (i.e., increased incidence of cognitive dysfunction, congenital heart defects, childhood leukemia, early-onset Alzheimer's disease (AD) and decreased incidence of solid tumors and cardiovascular disease). To test this hypothesis putative Hsa21-derived miRNA/mRNA targets must be identified. Computational miRNA/mRNA target programs remain the only source for rapid prediction of miRNA recognition sites harbored within the 3'-UTR of target mRNAs. For example, TargetScan analysis (TargetScan 5.0, <http://www.targetscan.org>),⁴³ predicted

that miR-99a could potentially interact with 40 putative mRNA targets; let-7c, 819 putative mRNA targets; miR-125b-2, 604 putative mRNA targets; miR-155, 281 putative mRNA targets and miR-802, 232 putative mRNA targets (Table 1). Although Hsa21-derived miRNAs may regulate a large number of mRNA targets (i.e., >1,695), it is still possible to prioritize and validate the importance of a predicted target in mediating the DS phenotype, given that target protein expression should be attenuated in DS individuals.

Hsa21-Derived miRNAs and Cognitive Dysfunction

All people with DS have a mild-to-moderate learning disability.⁷⁻⁹ The cognitive deficiencies in DS caused by the triplication of Hsa21 genes, is mainly a consequence of developmental perturbations. Brains of DS individuals are characterized

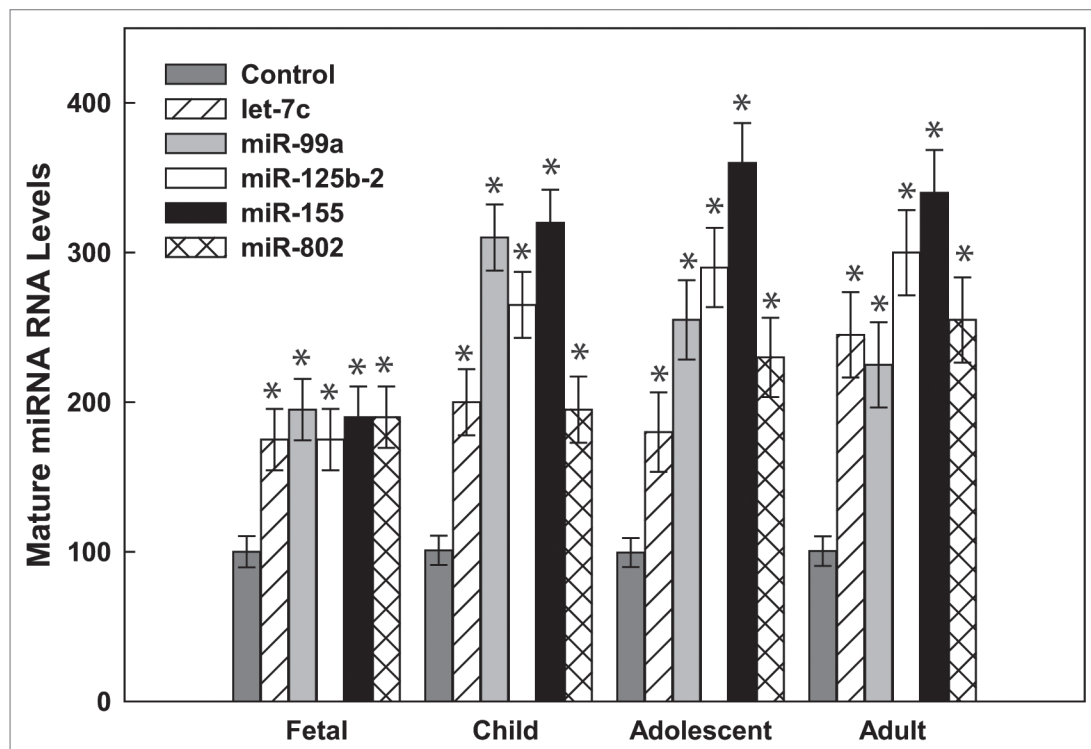


Figure 2. Hsa21-derived miRNAs are overexpressed in human DS pre-frontal cortex brain specimens. Mature Hsa21-derived miRNAs were quantified utilizing RT-PCR as previously described by our laboratory^{40,42,85,86} using total RNA isolated from human fetal (18–22 weeks of gestation), child (1–8 years), adolescent (9–19 years old) and adult (20–50 years old) prefrontal cortex specimens from control and DS (age- and sex-matched, $n = 3–5$) samples. Gene expression was calculated relative to 18S rRNA and the data are expressed as percent over control (variation between control samples was never greater than 5%), which was assigned a value of 100%. The error bars represent the average \pm S.E. of triplicate samples repeated in at least three independent experiments (* $p < 0.01$ DS vs. control).

Table 1. TargetsScan algorithm-predicted Hsa21-derived putative miRNA/mRNA targets

Hsa-21-derived miRNA	Potential human targets*	Number of targets containing at least <u>two</u> potential binding sites	Number of targets containing at least <u>three</u> potential binding sites	Number of targets containing at least <u>four</u> potential binding sites	Number of targets containing at least <u>five</u> potential binding sites
let-7c	819				
miR-99a	40				
miR-125b-2	604				
miR-155	281				
miR-802	232				
Total	1695	541	194	33	0

*Source: TargetsScan (April, 2009). The number of potential targets is dramatically dependent upon the algorithm utilized.

by neurological defects in cortex lamination and in cerebellar function (shape and volume).⁴⁴ Morphological and functional defects in DS individuals have also been found at the cellular level determined by alteration in neurogenesis, neuronal differentiation, myelination, dendritogenesis and synaptogenesis.^{45–48} Given that Ts21 results in the gene dosage overexpression of all five Hsa21-derived miRNAs in the brain,⁴⁰ these miRNAs may play a role in the developmental, morphological and

functional defects in the brain and ultimately the cognitive impairments observed in DS individuals. Therefore, the capability of each of the individual 1,695 putative Hsa21-derived miRNA/mRNA targets in playing a role in neurodevelopment were prioritized based on the following criteria: first, is the mRNA target expressed in the brain?; second, does the protein encoded by this mRNA target play a role in some aspect of neurodevelopment?; third, are there any studies linking mutations in the

gene of a given mRNA target to haploinsufficiency and problems in neurodevelopment (i.e., clinical relevance)?; and fourth, do any of these mRNA targets harbor multiple Hsa21-derived miRNA binding sites given that miRNA-mediated gene repression seems to be proportional to the number of miRNA recognition sites harbored in a given mRNA target (i.e., combinatorial miRNA inhibition)?^{49,50} Based on these criteria, we chose to investigate the transcription factor methyl-CpG-binding

protein (MeCP2) mRNA as a potentially important Hsa21-derived miRNA target given that its 3'-UTR harbored two miR-155 and -802 and single miR-125b and let-7c, putative recognition sites.⁴² Additionally, MeCP2 was a potential miRNA target since it is highly expressed in neurons and has been shown to play a role in neurogenesis,^{51,52} a process that is abnormal in DS individuals.^{4,7} Finally, MeCP2 was a provocative clinical target since Rett syndrome (RS) is a neurodevelopmental disorder caused by mutations in the MECP2 gene.^{51,52}

To validate that MeCP2 was indeed a true target of multiple Hsa21-derived miRNAs several independent experimental techniques were utilized. First, luciferase/target mRNA 3'-UTR reporter assays demonstrated that miR-155 and -802 could regulate MeCP2 protein expression levels by interacting with specific recognition sites harbored within its 3'-UTR.⁴² Second, miR-155 and -802 gain-of-function experiments demonstrated that MeCP2 mRNA and protein levels were attenuated and, conversely, miR-155 and -802 loss-of-function experiments demonstrated that MeCP2 mRNA and protein levels were augmented. These experiments suggested that MeCP2 mRNA was a target of both miR-155 and -802 and that these miRNAs markedly decreased MeCP2 expression by targeting MeCP2 mRNAs for degradation. Third, MeCP2 was under-expressed in DS brain specimens isolated from either humans or Ts65Dn mice (a widely used mouse model of DS^{53,54}), suggesting that MeCP2 may also be a target of Hsa21-derived miRNAs in vivo. Fourth, by manipulating the concentration of MeCP2 in cell culture, transcriptionally-activated and -silenced MeCP2 target genes,⁵⁵ *Creb1* and *Mef2c* were also modulated.⁴² Fifth, CREB1 and MEF2C, were also aberrantly expressed in DS brain specimens isolated from either humans or Ts65Dn mice. Finally, in vivo silencing of miR-155 or -802 with antagomirs^{56,57} (chemically modified, cholesterol-conjugated, single-stranded RNA analogs complementary to miR-155 or -802) resulted in the normalization of the appropriate miRNA, MeCP2, CREB1 and MEF2C expression in Ts65Dn mice.⁴² Taken together these results suggest that

Trisomy 21-induced, Hsa21-derived miR-155 and -802 overexpression directly inhibits MeCP2 expression which, in turn, leads to the aberrant expression of MeCP2-activated and -silenced target genes (e.g., *Creb1* and *Mef2c*) in vivo.⁴² Importantly, these studies support the hypothesis that improper repression (haploinsufficiency) of MeCP2, secondary to trisomic overexpression of Hsa21-derived miRNAs, contribute in part, to the abnormalities in the neurochemistry observed in the brains of DS individuals.

Hsa21-Derived miRNAs and the DS Phenotype

Given that our results suggest that Ts21 gene dosage overexpression of Hsa21-derived miRNAs, play a role in mediating the cognitive dysfunction observed in individuals with DS, it is tempting to hypothesize that the dysregulation of these miRNAs may also contribute to the other observed DS phenotypes including increased incidence of congenital heart defects, childhood leukemia, early-onset AD and decreased incidence of solid tumors and cardiovascular disease. While this hypothesis has not been directly tested there are a number of studies that substantiate the view that Hsa21-derived miRNAs may play a role, in part, in most of the observed DS phenotypes.

In support of the finding that Hsa21-derived miRNAs may play a role in the leukemias observed in DS children,⁶⁶ a subset of these miRNAs have been implicated in normal and pathologic hematopoiesis. For example, two of the most highly expressed miRNAs in pediatric AML (non-DS) were miR-125b and miR-99a.⁶⁷ Notably, miR-155 was also detected at elevated levels in the bone marrow of some patients suffering acute myeloid leukemia (AML).⁶⁸ Additionally, overexpression of miR-155 in hematopoietic stem cells in the mouse resulted in gross expansion of myeloid lineages in the bone marrow and peripheral blood at the expense of erythroid and lymphoid populations.⁶⁸ Moreover, transduction of miR-155 in the mouse macrophage RAW264.7 cell line induces dysregulation of a large number of genes, including 89 genes that were predicted to be targets of miR-155 on the basis of in silico analysis.

Importantly, ten of the predicted miR-155 target genes (e.g., PU.1 and C/EBP β) are involved in hematopoietic and myeloproliferative disorders.⁶⁹

To further corroborate our hypothesis that Hsa21-derived miRNAs may play a role in the early-onset Alzheimer's disease observed in DS individuals,⁷⁻⁹ miR-125b has been consistently reported to be upregulated in the cortex, hippocampus, cerebellum and the temporal lobe neocortex of AD (non-DS) affected tissue.⁷⁰⁻⁷² The dysregulation of miR-125b in AD may be particularly important as this miRNA is abundantly expressed in the normal brain and is involved in brain development.⁷³ Although experimentally validated miR-125b/mRNA targets that may play a role in mediating AD have not yet been identified, it is important to note that synapsin II, a neuron-specific phosphoprotein that selectively binds to small synaptic vesicles in the presynaptic nerve terminal, is a miR-125b predicted target (www.targetscan.org). Thus, the small increases in miR-125b observed in AD may have a bearing on the synaptic protein deficits previously observed in Alzheimer affected brains.^{74,75}

In further support of our hypothesis that Hsa21-derived miRNAs may play a role in the reduced incidence of solid tumors observed in DS individuals,¹⁰⁻¹⁴ other investigators have demonstrated that miR-99a, let-7c and miR-125b may act as tumor suppressors given that homozygous deletion (HD) of the miR-99a, let-7c and miR-125b-2 cluster is frequently associated with lung cancers.^{76,77} Additional evidence demonstrated that members of the let-7 family (which includes let-7c) were poorly expressed or deleted in human lung tumors and, importantly, one of the experimentally validated targets that was silenced by let-7 was the RAS oncogene.⁷⁸ Furthermore, many genes involved in cell proliferation were also silenced by let-7, including cyclins B1, E2, F, G₂ and cell division cycle 2 (CDC2), CDC25 and CDC34.⁷⁹ Interestingly, the exogenous delivery of let-7 prevented the formation of lung tumors from premalignant lesions and reduced tumors with activating RAS mutations.⁸⁰ Additionally, the under-expression of miR-125b-2 also seems to play an important role in lung

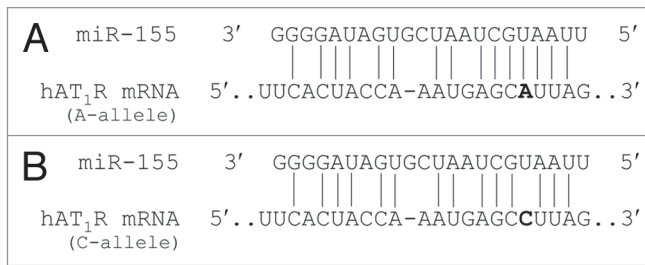


Figure 3. The human AT₁R +1166 A/C SNP occurs in the miR-155-binding site. (A) Complementarity between miR-155 and the hAT₁R 3'-UTR site targeted (70–90 bp downstream from the human AT₁R stop codon). The +1166 A/C SNP corresponds to the nucleotide 86 bp downstream from the human AT₁R stop codon (shown in bold print). The binding of miR-155 to the hAT₁R 3'-UTR target site fulfills the requirement of a 7 bp seed sequence of complementarity at the miRNA 5' end when the +1166 A-allele is expressed. (B) Complementarity between miR-155 and the human AT₁R 3'-UTR harboring the +1166 C-allele. If the +1166 C-allele is expressed, the seed sequence requirement would not be met and, as a consequence, it would be expected that human AT₁R expression would be elevated.

carcinogenesis, and one of its genetic targets includes the ERBB2 proto-oncogene that encodes the epidermal growth factor (EGF) receptor, which is highly expressed in carcinomas.^{81,82} Overexpression of miR-125a/b in an ERBB2-positive breast cancer cell line impaired cell growth and mobility capability,⁸² and frequent overexpression or amplification of ERBB2 was reported in ~20% of serous ovarian cancers.⁸³ Lastly, it has also been demonstrated that miR-99a, let-7c and miR-125b-2 expression is attenuated in prostate cancer.⁸⁴

Finally, to substantiate the finding that Hsa21-derived miRNAs may play a role in the reduced incidence of cardiovascular disease observed in DS individuals,¹⁵⁻²⁵ our laboratory has demonstrated that the human angiotensin II type 1 receptor (AT₁R) mRNA is a miR-155 target.^{85,86} AT₁R activation initiates a cascade of pathological events, including altered vascular tone, endothelial dysfunction, structural remodeling and vascular inflammation and it has been speculated that overproduction of the AT₁R contributes to the development of cardiovascular disease.⁸⁷ Interestingly, a silent single nucleotide polymorphism (SNP, +1166 A/C) in the human AT₁R gene has been associated with essential hypertension,^{88,89} cardiac hypertrophy,⁹⁰ aortic stiffness,⁹¹ myocardial infarction⁹² and increased oxidative stress levels in human heart failure.⁹³ Additionally, it has been demonstrated that patients with the human AT₁R CC genotype have enhanced

vascular reactivity⁹⁴⁻⁹⁶ and increased renovascular sensitivity to Ang II.⁹⁷ Initial miRNA experimental studies suggested that miR-155 could bind to the 3'-UTR of human AT₁R mRNAs and translationally repress the expression of this protein *in vivo*.⁸⁵ Computer alignment of the human AT₁R 3'-UTR sequence with the miR-155 sequence demonstrated that the +1166 A/C SNP occurs within the miR-155 cis-regulatory binding site (Fig. 3). The interaction between miR-155 and the human AT₁R 3'-UTR harboring the A-allele fulfills the seed sequence rule for miRNA/mRNA target interaction given that there is a 7 bp region of complementarity between the 5' end of miR-155 and the human AT₁R mRNA target site. In contrast, if a hAT₁R mRNA that harbors the +1166 C-allele is expressed, the complementary seed site is interrupted and the thermodynamics of the miRNA:mRNA duplex would be significantly altered.⁸⁶ Thus, the presence of the +1166 C-allele would decrease the ability of miR-155 to interact with the cis-regulatory site located in the hAT₁R 3'-UTR and, as a consequence, it would be expected that aberrantly high levels of the human AT₁R would be synthesized.

Given that the human AT₁R is an experimentally validated target of Hsa21-derived miR-155, it has been hypothesized that the AT₁R expression would be attenuated in individuals with DS due to the Ts21-induced gene dosage overexpression of miR-155. In support of this hypothesis, Ts21-isolated fibroblasts (which express

higher levels of miR-155), showed reduced protein levels of the human AT₁R when compared with that of euploid fibroblasts, which express lower levels of miR-155.³⁹ Taken together, these studies suggest that one possible explanation for the observed lack of cardiovascular disease in DS individuals may be due, in part, to the Ts21 gene dosage overexpression of miR-155 and the subsequent under-expression of the AT₁R.

Conclusion

Dysfunctional miRNA-mediated regulation has been implicated in the pathogenesis of many disease states. Given that Hsa21-derived miRNAs (miR-99a, let-7c, miR-125b-2, miR-155 and miR-802) may be involved in regulating either a single gene or sets of genes in common and divergent genetic networks, bioinformatics and systems biology tools will have to be employed to assist in the delineation of regulatory networks and feedback loops, by integrating signaling and transcription factors and identifying the presence of regulatory elements and links with other miRNAs. This line of investigation may result in the development of therapeutics that would benefit individuals with DS as well as individuals in the general population that suffer from aberrant brain development, congenital heart disease, childhood leukemia, Alzheimer's disease, cardiovascular disease and solid tumors.

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